

EVALUATION OF TWO-STAGE WEANING AND TRACE MINERAL INJECTION ON RECEIVING CATTLE GROWTH, BEHAVIOR, AND HEALTH

BY

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THESIS

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ABSTRACT

The objective was to evaluate effects of two-stage weaning and trace mineral injection on receiving cattle growth, behavior, and health. Two hundred ninety nine (163 heifers and 136 steers) Angus and Simmental \times Angus calves were utilized in a 2×2 factorial design. Calves were blocked by location and sex, and assigned to one of four treatments: 1) two-stage weaning and Multimin 90 (MULTIMIN USA, Fort Collins, CO) injection (**2MM**); 2) two-stage weaning and saline injection (**2SAL**); 3) abrupt weaning and Multimin injection (**AbtMM**); 4) abrupt weaning and saline injection (**AbtSAL**). On d -6, calves were weighed, inserted with Plastic Calf Weaner (Agri-Pro Enterprises of Iowa, Inc., Iowa Falls, IA) devices, received either Multimin 1ml/45.4kg BW) or saline (1ml/45.4kg BW) injections, and bled for plasma cortisol analysis. On d 0, calves were weighed, Plastic Calf Weaner devices were removed, and calves were shipped 272 km to Urbana, IL. Upon arrival, calves were weighed again and steer calves were bled for plasma cortisol analysis. All calves were weighed again on d 14, 28, 41, and 42. Additionally, steer calves were weighed on d 123 and 124. Steers were observed for behavior for the 2d following separation from the dam and checked daily for morbidity by the farm staff. There was no overall effect ($P \geq 0.23$) of treatment on steer body weight. Two-stage weaning decreased ($P = 0.01$) the percentage of BW shrink during shipping. There was no effect ($P \geq 0.14$) of weaning strategy or Multimin injection on overall steer ADG, however, 2MM steers tended ($P = 0.09$) to have an overall increased ADG compared to other treatment combinations. In the two weeks after separation, two-stage weaned calves had increased ADG ($P = 0.001$). There was an observed interaction ($P < 0.01$) on overall DMI where 2MM steers were increased compared to 2SAL but not different from AbtMM nor AbtSAL. There was an observed interaction ($P = 0.05$) on feed efficiency, where 2SAL steers were the most efficient. There was no effect ($P \geq 0.23$) of

treatment on cortisol levels. Two-stage weaning decreased ($P \leq 0.02$) the percentage of calves walking, standing, and vocalizing and increased ($P \leq 0.02$) the percentage of calves lying and eating. There was no effect ($P \geq 0.22$) of Multimin on any behavioral category. Two-stage weaning had no effect on overall growth, performance, or cortisol but decreased behavioral signs of stress. Multimin had no effect on calf BW, cortisol level, or behavior, but increased DMI in two-stage calves and tended to increase overall ADG.

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CHAPTER 1: REVIEW OF THE LITERATURE

INTRODUCTION

It is well known that the act of weaning in beef calves is a tremendously stressful period for the calves and accounts for great losses in performance. The most common weaning method is abrupt separation from the dam. Typically this is directly followed by transportation to another location where they are likely to experience nutritional, environmental and social stress in addition to the stress of separation. Calves vocalize repeatedly and spend more time walking which results in spending less time eating and resting (Veissier and Le Neindre, 1989). A common misconception is that it is best to separate the cow and calf far enough away so they cannot see or hear each other. However separating cows and calves by the greatest distance possible does not reduce their behavioral responses to traditional weaning (Neumann, 1977). In fact, providing fence-line contact for cows and calves by separating them into adjacent pastures actually decreases vocalizing and time spent walking, and increases time spent eating (Stookey et al., 1997). Due to the various stressors that occur at weaning, there is typically decreased nutrient intake. As a result, trace mineral levels decrease which may result in a deficiency of certain minerals. Trace mineral nutrition supports physiological functions related to growth, reproduction, and immunity in livestock (Underwood and Suttle, 1999). Although vaccination protocols may be utilized to protect against diseases at weaning, the calf immune system is still more vulnerable to infections. Therefore, weaning management, receiving practices, and trace mineral status can all play a major role in calf performance and a producer's profitability.

WEANING

Weight of calves at weaning is one of the most important economic drivers in profitability of a commercial cow-calf operation (Taylor and Field 1997; Myers, 1998) as calves that gain faster require fewer feed inputs per unit of gain, thus heavier weaning weights shortens time on feed for calves to reach a desired final weight (Neumann, 1977). Changes that are associated with weaning and shipping typically yield both behavioral and physiological responses of distress (Lefcourt and Elsasser, 1995; Stookey et al., 1997; Lay et al., 1998) leading to decreased DMI, which has a negative impact on weaning weights.

Under natural conditions, weaning in beef cattle involves the gradual decrease in milk supply from the mother, and a subsequent increase in the intake of solid food by the calf. When the calf is young, it is dependent on its dam's milk for nutrition. As the calf grows older it becomes increasingly able to fend for itself and thus less dependent on its mother for survival. At the same time the mother increasingly benefits from not allowing her calf to nurse so she can invest in future reproduction (Trivers, 1974). This results in a gradual reduction in the bond between the cow and calf (Martin, 1984) and the eventual weaning and separation of the cow-calf pair typically between 7 and 14 months of age (Enriquez et al., 2010).

Conventional beef production programs today expedite the weaning process by abruptly separating the cow and calf, usually at a much younger age than in natural circumstances (Hudson et al., 2010). Weaning in this case entails not only the abrupt secession of nursing between the cow and calf, but also the calf is denied social contact with the dam and other adult cattle (Stookey et al., 1997). This is done in order to allow the dam to regain body condition for the upcoming calf and thus increasing her productivity. Nutrient partitioning is very important for the reproductive performance of the cow. Once she is re-bred, the cow dedicates energy to

lactation to sustain her current calf at side while also utilizing energy to provide nutrients for her developing fetus (Freetly et al., 2006). The removal of the calf immediately lowers the dam's energy requirements shifting from lactation to maintenance (NRC, 2016).

In addition to reproductive advantages for the dam, weaning also allows the calf to become adjusted to a high concentrate diet. In order to receive more premiums and thus increase profitability, most feeders in the U. S. aim to produce Choice beef. The most widely used method to achieve this higher quality grade is the length of time cattle are fed a high concentrate diet (Myers, 1998). There are subsequent increases in marbling score and quality grade as the amount of time the animal spends on a high concentrate diet is extended (Campion et al., 1975). Therefore weaning and adjusting calves to a high concentrate diet prior to when they would be naturally weaned should increase carcass quality traits and premiums received.

AGE OF WEANING

There are several criteria that producers must consider when determining what age to wean their calves. In order to help improve reproductive efficiency of the cows, an important factor in deciding when to wean calves becomes the cow's BCS. Body condition at calving has been reported as a major determining factor in the re-establishment of cyclic ovarian activity in beef cows (Dunn et al., 1969; Whitman, 1975; Lowman et al., 1976; Dunn and Kaltenbach, 1980; Richards et al., 1986). Therefore, in an attempt to conserve a cow's BCS, without requiring as much nutritional inputs, producers wean calves earlier. A nursing calf and poor nutrition are two of the most important factors influencing the calving interval (Short et al., 1996).

One strategy that has been recommended to increase reproductive rates and thus help shorten the calving interval is early weaning, typically around 70-90 days of lactation (Enríquez

et al., 2011). Early weaning is an especially enticing management practice in years of drought (Ray et al., 1973; Sexten, 2001), when available feed is of poor quality, when cow's milk production is poor, when calves are nursing first calf heifers, or in herds that calve late (Ray et al., 1973). As a result, early weaning under these conditions has been shown to improve BCS of cows (Richardson et al., 1978). Weaning calves at this age allows the dams to devote more of the nutrients to reproduction instead of lactation, which in turn should increase conception rates (Sexten, 2001) and shorten the postpartum anestrous period (Bellows et al., 1974; Clemente et al., 1978). In a study by Meteer et al. (2013), early weaned calves (133 ± 21 d of age) had greater growing phase BW compared with calves weaned at a more typical age (100d after early weaned calves). In this study, early-weaned calves also had an increase in marbling scores. However early weaned calves had poorer BW gains and were less efficient than normal age weaned calves in the finishing phase. There have been both positive and negative effects of early weaning shown in multiple studies, therefore, producers must consider the needs of their own cow herd and program when deciding on an optimal age to wean calves.

WEANING STRATEGIES

In production, there are three main weaning strategies. The most widely used strategy is the traditional, abrupt weaning strategy. Traditional weaning involves completely separating the cow and calf abruptly allowing no physical or visual contact between the pair (Stookey et al., 1997; Hudson et al., 2010). This strategy is commonly used due to the fact that it requires less work. In order to try to decrease losses at weaning due to stress, poor performance and/or illness there have been two other weaning management strategies discovered. These two practices still involve the termination of nursing but allow for visual and/or physical contact between the cow

and calf to help mitigate the stress of weaning. The alternative weaning strategies help adjust the calves during weaning and may be less stressful than abrupt weaning.

The first of these two alternative strategies is fence-line weaning (Nicol, 1977), which involves separating the cow and calf into adjacent pens or pastures so they can still see and be in close proximity to their mothers while being prohibited from nursing and physical contact. Price et al. (2003) compared calves that were weaned via fence-line separation to calves that were abruptly weaned from their dams and found that providing fence-line contact reduced behavioral stress. Alternatively, Enriquez et al. (2010) compared calves that were weaned via fence-line contact to abruptly weaned calves and found that fence-line calves actually vocalized more than abruptly weaned calves and found no clear benefits in reducing weaning distress by utilizing the fence-line contact method.

The second alternative strategy is the two-stage technique that utilizes an anti-suckling nose clip that prohibits the calf from nursing but allows them to still have physical contact with their mothers (Haley et al., 2001). Haley et al. (2005) studied the effects of weaning calves in two stages using an anti-suckling device on their behavior and growth rate and found that two stage weaned calves vocalized less, spent less time walking, more time eating, and more time resting compared to abruptly weaned calves. In this study, although two-stage calves had greater ADG during the 7 d following separation, they had a decreased ADG when nursing was deprived and thus found no overall difference in ADG between the treatment groups. Enriquez et al. (2010) found no clear benefits in two-stage weaning compared to the traditional abrupt separation method. There was in fact a second distress response period after physical separation from the dam and a drop in ADG, which suggested that the response was distributed between the two stages of this weaning strategy.

BEHAVIORAL, PHYSIOLOGICAL AND PERFORMANCE RESPONSES TO STRESSES OF WEANING

The immediate responses to abrupt weaning of calves are predictable and occur for several days following separation (Haley et al., 2005). Calves spend more time walking and vocalizing and less time eating and resting when abruptly weaned (Veissier et al., 1989; Price et al., 2003). This decrease in intake and increase in activities can result in a subsequent loss in BW. Preweaning and preconditioning management strategies have been used to decrease stress at weaning (Thrift and Thrift, 2011). In a study by Haley et al. (2005), 116 two-stage weaned calves were fitted with an anti-sucking device for either 14 d or 3 d before separation and were compared with 74 control calves that were weaned abruptly. Abruptly weaned calves produced 41.9 calls/h, which was approximately 30 times more than the average of calves weaned in two stages (1.4 calls/h) on d 2 and 3 after separation. Also, calves weaned in two stages spent less time walking (14-d two stage = 34.8 min; 3-d two stage = 26.9 min) compared with control calves (146.3 min). Two stage weaned calves spent more time lying down and spent approximately 23% more time eating than the traditional control calves on d 2 and d 3 following separation. When evaluating performance, the abrupt weaned calves gained less weight during the first 8 d following separation than the two-stage treatment groups. However there was no difference in ADG from 0 d to 44 d between the calves weaned in two stages compared with the traditional calves. Overall the traditional calves had greater ADG than calves weaned in two stages. Price et al. (2003) found that fence-line weaned calves spent more time eating, more time lying down, less time walking and exhibited fewer frequencies of vocalization than abruptly weaned calves, particularly in the first 3 d following separation. Similarly, Stookey et al. (1997) found that abrupt, completely separated calves spent more time walking and vocalizing and less

time lying down and eating, particularly in the first 2 d post weaning than calves that were allowed fence-line contact with their dams. In the previously mentioned study by Price et al. (2003) the fence-line weaned calves not only had a greater ADG than the abruptly weaned calves in the first two weeks post weaning, but they continued to gain more throughout a ten week post wean period. This contrasts with the results of Haley et al. (2005) where the two-stage weaned calves had a greater ADG through the first 10 d post weaning than abruptly weaned calves but a lower overall ADG for the 44 d period compared with the abruptly weaned calves. Boland et al. (2008) compared the three weaning strategies of abrupt separation, fence-line contact and the use of nose-clip antisuckling devices. After separation on 0 d the abruptly weaned calves were observed to walk more and eat less compared to the fence-line and nose-clip calves and the abrupt calves had a reduced ADG compared to the other groups. They noted that the fence-line weaned calves had a greater ADG than the nose-clip calves.

Exposure to novel experiences and environmental conditions not only may induce physical stressors, but also psychological stressors (Grandin, 1997). Many common management practices, such as handling in a squeeze chute or transportation (Grandin, 1997), can be a source of psychological stress and be a causative factor of many clinical conditions in beef calves (Burton et al., 1995).

Cortisol concentration in an animal can be used as an indirect indicator to measure stress as stress increases the cortisol hormone in the blood serum (Peeters et al., 2011). Lay et al., (1998) studied the differences in plasma cortisol levels between restricted nurse calves (separated from their dams at 21 days of age and allowed to nurse once daily for 19 ± 3 d) and calves that were allowed to nurse ad libitum until weaning. Both treatment groups were weaned at 206 ± 4 days of age. Both treatments mean plasma cortisol concentration increased over time and were

highest at 1600 h on d 0 of weaning with the restricted nurse calves concentration being higher than the ab libitum calves. The restricted nurse calves experienced a greater increase in mean plasma cortisol concentration and elevated heart rates compared with the ad libitum calves in response to being restrained in a chute (Lay et al., 1998). In a study conducted by Hickey et al., (2003) it was found that abruptly weaned suckler calves are sensitive to the social stress associated with group disturbance and weaning as evidence of physiological changes in cortisol and noradrenaline concentration and weakening of immune function.

HEALTH

The biggest health challenge facing the cattle industry, and in newly weaned and received beef calves in particular, is bovine respiratory disease (BRD) due to its prevalence and economic ramifications (Duff and Galyean, 2007; Bolte et al., 2009; Schneider et al., 2009). Death losses ranged from 1.5 to 2.7 per 100 animals marketed in small feedlots (100 to 1,000 animals market annually) throughout the United States (USDA-APHIS, 1994). Of these deaths, two-thirds to three-quarters were attributed to respiratory disease (USDA-APHIS, 1994). Although expense of medicine and labor to treat BRD are drivers in its negative economic impact, feedlot performance and carcass quality also are negatively affected by BRD (Gardner et al., 1999) amplifying its economic impact. In 1999, 97.4% of feedlots within 12 states reported an overall BRD incidence of 14.4% (USDA-APHIS, 2000a). Treatment costs for BRD averaged \$15.57 per treated animal and costs are significantly higher when labor, isolation, time on feed and mortality are considered (USDA-APHIS, 2000a). When marketing cattle, Fulton et al. (2002) reported that calves treated for BRD once returned \$40.64 less, calves treated twice returned \$58.35 less, and calves treated 3 or more times returned \$291.93 less than calves that were never treated for BRD. Annually, BRD has been reported to cost the industry \$750 million in losses (Griffin, 1997).

Many types of infections are characterized as BRD with each having its own causes, clinical signs and economic implications (Snowder et al., 2006). Microbial causes for BRD include bacterial (*Mannheimia haemolytica*, *Pasteurella multocida*, *Haemophilus somnus*) and viral (infectious bovine rhinotracheitis, bovine viral diarrhea, bovine respiratory syncytial, and parainfluenza type 3; Ellis, 2001). There are many sources of stress that lead to a high incidence of BRD in newly weaned and received calves. Stresses associated with weaning, shipping and processing lightweight feedlot cattle negatively impact the immune system (Blecha et al., 1984) during a period of time when the animal is often exposed to infectious agents as they are commingled from various sources as a result of marketing practices (Galyean et al., 1995; Snowder et al., 2006; Step et al., 2008; Schneider et al., 2009). At the same time, these stressors typically result in subsequent low feed intake, averaging approximately 1.5% of BW during the first 2 wk after arrival of the newly received calves (Galyean and Hubbert, 1995). This decrease in feed intake may further impair immune function due to a decrease in trace mineral status (Cole, 1996). The act of excessive vocalization at weaning can also increase susceptibility of the respiratory tract to infection and potentially leading to BRD (Loerch and Fluharty, 1999).

There are different management practices that producers can utilize in an attempt to decrease the incidence of BRD among recently weaned and received calves. Most feedlot producers believe that preconditioning cattle is somewhat to extremely beneficial in decreasing morbidity and mortality in calves weighing less than 318 kg (USDA-APHIS, 2000a), however only 32.4% of the feedlots surveyed received information about the previous history of the calves “always or most of the time” (USDA-APHIS, 2000b). Preconditioning programs are utilized to decrease stresses associated with weaning, enhance the immune systems of calves and thus ease the transition to the next phase of production (NRC, 2016). Typically, preconditioning

programs ensure that the calves have been weaned for a certain amount of time (usually 30 to 45 d), vaccinated (with clostridial and viral vaccines), treated with anthelmintic, castrated, dehorned and accustomed to feed bunks and water troughs before being transported to a feedlot (Duff and Galyean, 2007; Thrift and Thrift, 2011). Preconditioned calves were more profitable in the feedlot, primarily due to greater daily gain, improved efficiency, decreased medication cost and decreased mortality compared with non-preconditioned calves (Cravey, 1996).

TRACE MINERALS

Trace minerals are essential to the health and growth of cattle and aid in supporting physiological functions related to skeletal development, immunity and reproduction in livestock (Underwood and Suttle, 1999). Supplemental trace minerals can be given free choice or included in the diet in the form of inorganic mineral or organic minerals. Trace minerals can also be supplemented via an intramuscular or subcutaneous injection. Actual mineral requirements of stressed calves does not seem to be greater than non-stressed calves (Cole 1993), however stressed calves may have trouble meeting requirements due to decreased DMI. During times of weaning and shipping, which often occur together, the NRC (2016) recommends increasing dietary levels of TM to approximately 150% of non-stressed cattle requirements to maintain adequate levels of TM being consumed.

Inorganic minerals generally are cheaper in cost, however they are typically less bioavailable for the ruminant to utilize as an organic form. An animal's ability to convert inorganic trace minerals to organic biologically active forms determines how well it can utilize those inorganic trace minerals (Spears, 1996). Therefore variations in performance must be considered when deciding if inorganic minerals or organic minerals are more cost effective (Stewart, 2010).

The development and reason of interest behind organic trace minerals, or chelated minerals, is the theory that they are more bioavailable and more similar to forms that occur in the body than inorganic sources of trace minerals (Spears, 1996). In order to be classified as a chelate the chelating agent or ligand must: 1) contain at minimum two functional groups (hydroxyl, amino, oxygen, nitrogen) that are all capable of donating a pair of electrons that combine with a metal and 2) form a heterocyclic ring structure with metal (Kratzer and Vohra, 1986). The metal would be protected from forming complexes with other dietary components that inhibit absorption if the metal chelate is stable in the digestive tract, resulting in greater absorption (Spears, 1996). In particular, when Zn and Cu are complexed with proteins or amino acids, such as lysine or methionine, they tend to be more effective than inorganic forms of trace elements when given to stressed cattle (McDowell, 1996).

One strategy that has been implemented to mitigate the variation in intake of free choice and diet supplemented trace minerals is the use of supplementing these trace minerals via an intramuscular or subcutaneous injection (injectable trace minerals [ITM]). The advantage seen with ITM, compared with more traditional forms of trace mineral supplementation, is that each animal receives the targeted amount of trace minerals (Arthington et al., 2014). Newly weaned and received cattle are often stressed and have a decreased DMI (Ceciliani et al., 2012), thus limiting TM intake and likely decreasing the overall TM status. Obtaining adequate levels of essential trace minerals can be assured through ITM. A proper vaccination program, along with becoming accustomed to bunk-style feeding and water troughs can help alleviate morbidity in the feedlot due to BRD (Duff and Galyean, 2007), the greatest and most economically devastating health challenge facing the US beef cattle industry (Duff and Galyean, 2007; Bolte et al., 2009). In conjunction with the previously mentioned practices, administering injectable TM

along with vaccinations can increase neutralizing antibody titers in response to vaccinations (Arthington and Havenga, 2012). This suggests that TM injection prior to weaning and shipping is an ideal time to improve the TM status of calves. Arthington et al. (2014) examined the effects of ITM on performance and trace mineral status of beef calves prior to, during and post weaning. In calves prior to weaning they found that ITM increased the trace mineral status but did not have an effect on ADG. After weaning and shipping they found that although trace mineral status was again greater for ITM calves, ITM calves actually had a lesser ADG compared to calves injected with saline. After weaning and shipping calves were evaluated in a 177 d study and it was found that ADG and humoral response to novel antigens was greater in ITM calves compared to saline injected calves. Similarly, Genther-Schroeder and Hansen (2015) found that during a preconditioning period prior to shipping stress, calves injected with ITM had increased trace mineral concentrations but ADG and BW shrink from shipping was not different from calves injected with saline. Like the previously mentioned study they found that ITM calves had a decreased ADG during the 14 d period after transit and shipping stress compared to saline injected calves. Roberts et al. (2016) conducted a study to test the effect of ITM on health, performance, and vaccine response of newly received feedlot cattle and found that ITM did not improve the performance or morbidity of their cattle when instances of morbidity incidences were already low. They did, however, find the Bovine Viral Diarrhea Virus (BVDV)-specific antibody response to a respiratory vaccine was greater on d 14 for ITM. Although there have been varying reports of the effects of ITM on calf health and performance at weaning, it is clear that it has the ability to correct the trace mineral status of calves that are deficient. This in turn can have positive impacts on the well-being of beef calves at times of stress. Due to the fact that

it is difficult to know the mineral status of cattle at any time, ITM can offer peace of mind to producers by ensuring proper levels of trace minerals.

COPPER

Although the requirements for Cu in beef cattle diets may vary, the recommended concentration is 10 mg Cu/kg (NRC 2016). Molybdenum and S have an antagonistic effect on Cu and when Mo and S exceed 2 mg Mo/kg dietary DM and 0.25%, Cu requirements will increase in beef cattle (NRC 2016). Thiomolybdates are formed in the rumen by an interaction between molybdate and sulfide (Suttle, 1991). Thiomolybdates can cause Cu to be tightly bound to plasma albumin and unavailable for biochemical functions, and potentially directly inhibit certain Cu-dependent enzymes (NRC 2016). The feedstuff that serves as a source of Cu can alter the effects of S and Mo. Breed of cattle can also affect Cu requirements (NRC 2016).

Gooneratne et al. (1994) saw that Simmental cattle excrete more Cu in the bile than Angus cattle and Ward et al. (1995) found that plasma Cu concentrations were higher in Angus cattle than Simmental and Charolais cattle suggesting that breed of cattle may have an effect on copper requirements and susceptibility to Cu deficiency (NRC, 2016). Copper deficiency in cattle can cause decreased growth, anemia, cardiac failure, fragile bones, diarrhea, depigmentation and changes in growth and appearance of hair and negative effects on reproduction (NRC, 2016). Copper deficiency decreases the ability of bovine neutrophils to kill phagocytized microorganisms, but the effects of a Cu deficiency on specific immune responses of cattle have not been consistent (Ward and Spears, 1999). In a study by Ward and Spears (1999), copper status did not have an effect on immune response during the receiving phase, but Cu supplementation did alter the immune response during the growing phase suggesting that Cu deficiency alters cattle's immune response only after extended periods. Excess concentrations of

Cu in the diet from supplementation or from feeds that have been tainted with extra Cu from industrial and agricultural sources, such as different by-products used for feed, can lead to Cu toxicity (NRC, 2016). When the liver builds up with Cu and then is released it can cause jaundice, hemolysis, necrosis and death (NRC, 2016).

SELENIUM

The NRC (2016) suggests that beef cattle should consume 0.1 mg Se/kg dietary DM daily. Boyne and Arthur (1981) found that cattle fed a Se deficient diet had neutrophils that were less able to kill *C. albicans* suggesting a decreased neutrophil activity and poorer response to infectious pathogens. Reffett et al. (1988) saw that calves fed a Se deficient diet and inoculated with IBRV had reduced antibody titers compared to calves fed a Se sufficient diet inoculated the same way. This too suggests a reduced humoral immune response in cattle that are Se deficient. Generally, organic forms of selenium are absorbed more readily by animals than inorganic forms of selenium (Ammerman and Miller, 1975). The functions of Se and vitamin E are antagonistic, so consequently a diet that is low in vitamin E can subsequently increase the amount of Se required (Miller et al., 1988). Environmental and dietary stressors can also increase Se requirements (NRC, 2016). Selenium deficiency can cause nutritional muscular dystrophy in younger calves, and reproduction issues such as infertility, abortions, stillbirths or retained placenta. Selenium deficient cattle may also show general unthriftiness and a decrease in BW (Underwood and Suttle, 1999). In a study by Spears et al. (1986) calves that were deficient in Se had reduced weaning weights and an increase in mortality rates. The maximum concentration of Se suggested by NRC (2005) was set at 2 mg/kg dietary DM, however many research trials have been completed since then that suggest that cattle are able to tolerate greater concentrations of Se (NRC, 2016).

ZINC

In order to meet most beef cattle's Zn requirements in most situations, NRC (2016) suggests diets should contain 30 mg of Zn/kg dietary DM. There have been varying responses on performance indicators to supplemental Zn fed to beef cattle (Perry et al., 1968; Spears and Kegley, 2002). Cattle that are deficient in Zn may experience reduced growth, reduced feed intake, impaired reproduction, excessive salivation, swollen feet, hair loss, and skin lesions that are typically more pronounced on the legs, neck, head, and around the nostrils (Spears, 1995).

Calves with a genetic disorder that impairs their ability to absorb Zn have been observed to have thymus atrophy and impaired immune response (Perryman et al., 1989; Spears, 1995). Zinc toxicity occurs at concentrations of Zn much greater than the suggested required concentration and a suggested maximum concentration of Zn is 500 mg/kg (NRC, 2016). Zinc is an important trace mineral for the normal development and functioning of the immune system (NRC, 2016). Cattle's immune system response to a number of harmful pathogens causes a drop in the concentration of Zn in the blood, resulting in an impairment of the body's ability to fight off these pathogens and a subsequent increase in mortality (McDowell, 2002).

MANGANESE

The recommended concentration of Mn in the diet of beef cattle varies depending on stage of production. Mn plays a greater role in reproduction than skeletal development and growth and the body also has been found to require more Mn for skeletal development than growth (NRC, 2016). Therefore, it is recommended to supplement Mn at a rate of 20 mg of Mn/kg diet for young and finishing cattle and 40 mg of Mn/kg for breeding age cattle used for reproduction (NRC, 2016). Rojas et al. (1965) found that cows deficient in Mn required a greater number of services to conceive than control cows. In addition, there were also issues with calves

born to the cows deficient in Mn. Calves from cows fed low Mn rations were born with enlarged joints, twisted legs, stiffness, weaker humerus, and were observed to be physically weak.

SUMMARY

It is well known that times of heightened stress, such as weaning, can cause losses in production and profit for cattle producers. Researchers have long been attempting to minimize stress at weaning and thus subsequent losses occurred. Although there has been some varying results of two-stage weaning, it has typically been found to decrease instances of behavioral stress. The key roles trace minerals play in beef cattle production is also well known and although there has been varying results of the effects of ITM in beef calves at weaning, it is clear that it has the ability to correct the trace mineral status of calves that are or are border-line deficient. Identifying the best practices producers can utilize at weaning in order to minimize losses has been the goal of many researchers and many studies have been conducted evaluating the effects of different weaning strategies and altering trace mineral status' separately. Perhaps evaluating these two practices in conjunction with one another will offer more insight as to how to best decrease losses at weaning due to stress and ultimately increase the cow-calf producer's profit.

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CHAPTER 2: EVALUATION OF TWO-STAGE WEANING AND TRACE MINERAL INJECTION ON RECEIVING CATTLE GROWTH, BEHAVIOR, AND HEALTH

ABSTRACT

The objective was to evaluate effects of two-stage weaning and trace mineral injection on receiving cattle growth, behavior, and health. Two hundred ninety nine (163 heifers and 136 steers) Angus and Simmental \times Angus calves were utilized in a 2×2 factorial design. Calves were blocked by location and sex, and assigned to one of four treatments: 1) two-stage weaning and Multimin 90 (MULTIMIN USA, Fort Collins, CO) injection (**2MM**); 2) two-stage weaning and saline injection (**2SAL**); 3) abrupt weaning and Multimin injection (**AbtMM**); 4) abrupt weaning and saline injection (**AbtSAL**). On d -6, calves were weighed, inserted with Plastic Calf Weaner (Agri-Pro Enterprises of Iowa, Inc., Iowa Falls, IA) devices, received either Multimin 1ml/45.4kg BW) or saline (1ml/45.4kg BW) injections, and bled for plasma cortisol analysis. On d 0, calves were weighed, Plastic Calf Weaner devices were removed, and calves were shipped 272 km to Urbana, IL. Upon arrival, calves were weighed again and steer calves were bled for plasma cortisol analysis. All calves were weighed again on d 14, 28, 41, and 42. Additionally, steer calves were weighed on d 123 and 124. Steers were observed for behavior for the 2d following separation from the dam and checked daily for morbidity by the farm staff. There was no overall effect ($P \geq 0.23$) of treatment on steer body weight. Two-stage weaning decreased ($P = 0.01$) the percentage of BW shrink during shipping. There was no effect ($P \geq 0.14$) of weaning strategy or Multimin injection on overall steer ADG, however, 2MM steers tended ($P = 0.09$) to have an overall increased ADG compared to other treatment combinations. In the two weeks after separation, two-stage weaned calves had increased ADG ($P = 0.001$). There was an

observed interaction ($P < 0.01$) on overall DMI where 2MM steers were increased compared to 2SAL but not different from AbtMM nor AbtSAL. There was an observed interaction ($P = 0.05$) on feed efficiency, where 2SAL steers were the most efficient. There was no effect ($P \geq 0.23$) of treatment on cortisol levels. Two-stage weaning decreased ($P \leq 0.02$) the percentage of calves walking, standing, and vocalizing and increased ($P \leq 0.02$) the percentage of calves lying and eating. There was no effect ($P \geq 0.22$) of Multimin on any behavioral category. Two-stage weaning had no effect on overall growth or cortisol but decreased behavioral signs of stress. Multimin had no effect on calf BW, cortisol level, or behavior, but increased DMI in two-stage calves and tended to increase overall ADG.

INTRODUCTION

Most beef operations wean by abruptly separating the cow and calf, usually at a much younger age than in a natural situation (Hudson et al., 2010). The results of abrupt weaning are well documented and consistent, as cows and calves spend more time walking and vocalizing and less time resting or eating (Veissier and Le Neindre, 1989). Excessive vocalizations can increase the respiratory tract's susceptibility to infectious pathogens that can lead to bovine respiratory disease (**BRD**; Loerch, and Fluharty, 1999), considered the biggest health challenge facing the cattle industry (Duff and Galyean, 2007).

Alternative weaning strategies such as fence line weaning (Nicol, 1977) and two-stage weaning with a plastic nose weaning clip (Haley et al., 2001) have been investigated and both strategies positively influence behavior (Price et al., 2003 and Haley et al., 2005); however, impacts on performance are inconclusive.

Trace minerals (TM) are essential to the health and growth of cattle (Underwood and Suttle, 1999). Newly weaned and received cattle are often stressed and have a decreased DMI

(Ceciliani et al., 2012), which could limit TM intake. Injectable tract minerals [**ITM**] ensure each animal receives the targeted amount of TM (Arthington et al., 2014). Performance responses to ITM at times of weaning and receiving are variable (Richeson and Kegley, 2011; Arthington et al., 2014; Genther-Schroeder and Hansen, 2015). Richeson and Kegley (2011) reported that calves that received ITM had reduced morbidity rates.

To our knowledge, no work has evaluated the interaction of weaning strategy and ITM. Therefore, our objective of this trial was to study the interaction between weaning strategies and ITM on receiving calf performance, behavior, and health. The hypothesis of this study was trace mineral injection would be more advantageous in abruptly weaned calves due to greater stress and lower feed intake upon arrival.

MATERIALS AND METHODS

Animals, Experimental Design, and Treatments

Two hundred ninety nine Angus and Simmental × Angus calves (d -6 BW = 198 ± 26 kg) (146 calves; 85 heifers and 61 steers) from the Orr Agricultural Research and Demonstration Center (**ORC**) in Baylis, IL and (153 calves; 78 heifers and 75 steers) from the University of Illinois Beef Cattle and Sheep Field Research Laboratory (**URB**) in Urbana, IL were utilized to evaluate the effects of two-stage weaning and trace mineral injection on receiving cattle behavior and growth. Experimental procedures followed those approved by the University of Illinois Laboratory Animal Care Advisory Committee's protocol 15143.

Experimental Design

A randomized complete block design with a two × two factorial arrangement of treatments was used. Calves were blocked by location and assigned to one of four treatments: 1) two-stage weaning and Multimin injection (**2MM**); 2) two-stage weaning and saline injection

(**2SAL**); 3) abrupt weaning and Multimin injection (**AbtMM**); 4) abrupt weaning and saline injection (**AbtSAL**).

Calf management and data collection

Calves at URB and the ORC received Calf Guard (Zoetis, Kalamazoo, MI) and Ecolizer + C20 (Elanco Animal Health, Greenfield, IN) at birth. Two to three days after birth, calves at both facilities were administered BO-SE (Merck Animal Health, Madison, NJ), Bovi-Sera (Colorado Serum Company, Denver, CO), Inforce 3 (Zoetis, Kalamazoo, MI). At this time calves at URB also received Vitamin A and D, and a metaphylactic treatment of Excede (Zoetis, Kalamazoo, MI). Calves (mean \pm SD, 126 ± 19 days of age) at both facilities received BoviShield Gold FP5 VL5 HB (Pfizer, New York, NY), Autogenous Mycoplasma Vaccine (New Port Laboratories, Worthington, MN), Covexin 8 (Merck Animal Health, Madison, NJ), and all bulls were banded for castration. Calves (175 ± 19 days of age) at ORC also were administered One Shot Ultra 8 (Zoetis, Kalamazoo, MI). Boosters of BoviShield Gold FP5 VL5 HB and Autogenous Mycoplasma Vaccine were given to calves at both facilities. In addition to these vaccines, calves at ORC also received UltraChoice 8 (Zoetis, Kalamazoo, MI), Inforce 3, and Eprinex (Merial, Duluth, GA).

On d -6, calves were weighed, Plastic Calf Weaner devices were inserted, and calves received either Multimin (1 ml/45.4 kg of BW) or saline (1 ml/45.4 kg of BW) injections. On d 0, calves were weighed, Plastic Calf Weaner devices were removed, and calves were shipped 271.98 km to Urbana, IL. Calves from Urbana were shipped via commercial trucking for an equal distance as ORC cattle so that calves from both facilities were subject to an equal amount of shipping time and stress. Upon arrival, calves were weighed again. Pre-trucking and arrival weights were used to calculate a percent body weight shrink for each calf. Calves were sorted by

location, sex and treatment and randomly assigned to pens [5-7 steers per pen (4.88m × 10.36m) and 6-8 heifers per pen (4.88m × 4.88m)] on d 0 after being processed through the working facility. Calves were housed in four barns (heifers in two separate barns and steers in two separate barns). Barns were constructed of a wood frame with a ribbed metal roof and with siding on the north, west, and east sides. The south side of the barn was covered with polyvinyl chloride-coated 1.27 by 1.27 cm wire mesh bird screen and equipped with retractable curtains for wind protection. Pens had slatted concrete floors covered by interlocking rubber matting. Weaning method was separated by barn to minimize influence of other treatment on behavior. Calves were fed in 4.27m concrete feed bunks. Day 0 to d 42 is referred to as the “Receiving Period”. Calves were offered ab libitum access to a receiving diet (45% silage, 25% chopped hay, 20% distiller’s grain, 10% supplement) on d 0 to d 6. From d 7 to d 42 calves were offered ab libitum access to a transition diet (Table 1; 55% silage, 15% chopped hay, 20% modified distillers grains, 10% supplement). Heifers were off trial at d 42. From d 43 to d 53 steers were offered ab libitum access to (40% silage, 10% chopped hay, 20% modified distillers grains, 10% supplement, 20% high moisture corn). From d 54 to the end of the trial steers were offered ab libitum access to a growing diet consisting of (15% silage, 20% modified distiller’s grain, 55% high moisture corn, 10% supplement. Calves were weighed again on d 14, 28, 41, and 42. Final receiving BW for all calves was determined by averaging a 2-d consecutive weight on d 41 and 42. Steers only were weighed on d 123 and d 124 for a final 2-d off weight to determine growing period performance amongst steer calves. The “Growing Period” was considered to be from d 42 to d 124 and the “Overall” was considered d 0 to d 124. Incidences of morbidity were recorded by animal care personnel daily throughout the duration of the trial.

Feed and Plasma Analysis

Feed ingredient samples were collected on d 14, 28, 42, 70, 98, and 124 of the trial. Feed samples were dried (3 d at 55°C) and then ground using a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Ground forage and feed ingredient samples were analyzed for CP (Leco TruMac, LECO Corp., St. Joseph, MI) and fat (using Ankom Technology method 2; Ankom XT Fat Analyzer, Ankom Technology, Macedon, NY), ADF and NDF (using Ankom Technology methods 5 and 6, respectively; Ankom Technology), CP (Leco TruMac, LECO Corp.), fat (method 2; Ankom Technology). Feed bunks were cleaned to measure feed refusal in each barn on d 7, 14, 20, and 43 of the trial. Feed refusals were then weighed and aliquots were taken to be dried in order to determine dry matter content.

On d -6 and d 0, 10 mL of blood was collected on steer calves via jugular venipuncture into K₂ EDTA blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) before being centrifuged at $1,300 \times g$ for 20 min at 5°C. Plasma was separated and transferred into 1.5 mL aliquots and stored at -20°C for cortisol analysis. Plasma concentrations of cortisol were determined using a single chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Intra-assay CV for the analyses of cortisol was 1.9%.

Behavior

Behavior was observed on d 1 and d 2, in 4 h time blocks rotating every h, exclusively on steer calves (24 total pens divided among two separate barns). Cattle were fed at 0700 and calf behavior was observed from 0800 to 2000 on both days. Behavior assessment was taken in 10 minute intervals. The number of steers lying, standing, walking, and eating were recorded and activities were not mutually exclusive (steers could be standing and eating). On a rotating basis (4 pens per 10 minute interval), pens were sampled for vocalizations (total number of

vocalizations in 2 minutes). Any audible vocal sound that could be attributed to a specific calf was counted as a vocalization. Undergraduate and graduate student volunteers from the University of Illinois Department of Animal Sciences were used to observe cattle behavior and served in 4 h shifts. Students were blind to the location of origin and treatment of the steers.

Statistics

Pen was considered the experimental unit for all calf performance and behavior characteristics. Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). For growth performance, intake, and efficiency data, the least squares means were used to separate treatment means. The statistical model included weaning strategy, trace mineral injection, the interaction of weaning strategy and trace mineral injection, location, and sex as fixed effects and pen as a random effect. For behavioral data, data was analyzed on a pen basis and the model included weaning strategy, trace mineral injection, the interaction of weaning strategy and trace mineral injection, and location as fixed effects. Treatment effects were considered significant at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS

Calf Performance

Calf performance data are presented in Table 2. There was no weaning strategy by ITM interaction ($P \geq 0.29$) for BW at any time. Similarly, neither weaning strategy ($P \geq 0.23$) nor ITM ($P \geq 0.38$) affected BW at any time. There was no weaning strategy by ITM interaction ($P = 0.17$) nor ITM effect ($P = 0.67$) on shrink. However, there was a decrease ($P = 0.01$) in shrink for 2-stage weaned calves compared to abruptly weaned calves. When evaluating ADG, there was a tendency for a weaning strategy by ITM interaction ($P = 0.10$) from d 0 to d 42 and also a tendency for an interaction ($P = 0.06$) from d -6 to d 124 for steer calves. Steer calves also had a

trend for an interaction ($P = 0.09$) from d 0 to d 124 for ADG. There was no weaning strategy by ITM interaction ($P \geq 0.13$) for ADG from d -6 to d 0, d 0 to d 14, d 14 to d 28, d 28 to d 42, d 42 to d 124, nor d -6 to d 42. From d -6 to d 0, 2-stage weaning decreased ($P < 0.01$) ADG. However, 2-stage weaned calves had increased ($P = 0.001$) ADG from d 0 to d 14 compared to abruptly weaned calves. From d 14 to d 28 2-stage weaned calves had a decreased ($P = 0.05$) ADG compared to abruptly weaned calves. Two-stage weaned calves had a decreased ($P = 0.02$) ADG from d -6 to d 42 compared to abruptly weaned calves as well. There was no weaning strategy effect ($P \geq 0.38$) on ADG for the remainder of the time periods. Multimin tended ($P = 0.06$) to increase ADG compared to saline injected calves from d 0 to d 124, but there was no ITM effect ($P \geq 0.14$) for the remainder of the time periods.

Calf intake and feed efficiency data can be found in Table 3. For the Receiving Period, there was no weaning strategy by ITM interaction ($P \geq 0.14$) for DMI. For DMI during the growing period from d 42 to d 124, there was a weaning strategy by ITM interaction ($P = 0.01$); 2MM steers had the greatest DMI, AbtMM and 2SAL steers had the lowest DMI, and AbtSAL steers were intermediate and not different. For overall DMI, there was also a weaning strategy by ITM interaction ($P < 0.01$); 2MM steers again had the greatest DMI, 2SAL had the lowest DMI, and AbtMM and AbtSAL steers were intermediate and not different. For overall efficiency from d 0 to d 124, there was a weaning strategy by ITM interaction ($P = 0.05$); 2SAL steers had the greatest G:F, AbtSAL steers had the lowest G:F, and AbtMM and 2MM steers were intermediate and not different. There were no weaning strategy by ITM interactions ($P \geq 0.59$) for feed efficiency during any time period throughout the receiving period from d 0 to d 42. Feed efficiency for the growing period from d 42 to d 124 showed a trend ($P = 0.06$) for a weaning strategy by ITM interaction. The effects of weaning strategy on DMI revealed that 2-stage

weaning decreased ($P = 0.01$) DMI from d 14 to d 27 and tended ($P = 0.09$) to decrease DMI from d 0 to d 41. Weaning strategy had no effect ($P \geq 0.15$) on DMI from d 0 to d 14, d 28 to d 42, d 42 to d 124, nor d 0 to d 124. Two-stage weaning decreased ($P = 0.01$) feed efficiency from d 0 to d 14. However, 2-stage increased ($P = 0.04$) feed efficiency from d 28 to d 42 and tended ($P = 0.08$) to increase from d 0 to d 42 during the receiving period. Weaning strategy had no effect ($P \geq 0.12$) on feed efficiency from d 14 to d 28, d 42 to d 124, nor d 0 to d 124. When evaluating the effects of ITM on DMI and feed efficiency, Mutimin increased ($P = 0.04$) DMI from d 28 to d 42 and tended to increase DMI from d 0 to d 42. There was no ITM effect ($P \geq 0.14$) on DMI from d 0 to d 14, d 14 to d 28, nor d 42 to d 124. There was no ITM effect ($P \geq 0.15$) on efficiency at any time throughout the study.

Cortisol

Steer cortisol analyses are shown in Table 4. There was no weaning strategy by ITM interaction ($P \geq 0.23$) among treatment groups. Weaning strategy did not have an effect ($P \geq 0.53$) on cortisol levels. There was no ITM effect ($P \geq 0.36$) on cortisol levels.

Behavior and Vocalizations

Table 5 shows the effects of weaning strategy and Multimin inclusion on calf behavior. There was a weaning strategy by ITM interaction ($P = 0.01$) on the percent of steers walking on d2; AbtMM steers walked the most, AbtSAL steers were intermediate, and 2MM and 2SAL steers walked the least. There was also a tendency ($P = 0.09$) for an interaction on the percent of steers walking on d 2 as well. There were no weaning strategy by ITM interactions ($P \geq 0.28$) for the percent of steers standing and lying on either d 1 or d 2 nor percent of steers walking and eating on d 1. There were no weaning strategy by ITM interactions ($P \geq 0.29$) on the number of vocalizations on either day. On d 1, weaning strategy had an effect ($P \leq 0.01$) on the percent of

steers standing, lying, eating, and walking were a greater percentage of 2-stage steers were recorded eating and lying and a lesser percentage of 2-stage steers were recorded standing and walking. On d 2, there was a weaning strategy effect ($P \leq 0.02$) on the percentage of steers standing, lying, and eating. A greater percentage of 2-stage steers were recorded standing and eating and a lesser percentage of 2-stage steers were recorded lying. On both days where behavior was observed, 2-stage steers also vocalized significantly less ($P < 0.01$) than abruptly weaned steers. Injectable trace mineral had no effect ($P \geq 0.22$) on the percent of steers standing, lying and eating on either d 1 or d 2 and had no effect on the percent of steers walking on d 1 ($P = 0.61$). There was no ITM effect ($P \geq 0.36$) on the number of vocalizations on either d 1 or d 2.

DISCUSSION

WEANING STRATEGY * ITM INTERACTION

There were no weaning strategy by ITM interactions on BW throughout the entire study nor percentage of BW shrink. There was a tendency for a weaning strategy by ITM interaction for ADG from d 0 to d 42, d -6 to d 124, and from d 0 to d 124 where 2MM calves' ADG was increased for each period. This may partially be explained by the fact that 2MM steers had an increase in DMI from d 0 to d 124 and also from d 42 to d 124. Studies by Arthington et al. (2014) and Genther-Schroeder and Hansen (2015) showed that calves that received ITM actually had a decrease in ADG compared to calves receiving saline injections for 14 d following a period of stress. Researchers in the current study did not find this; however ITM did tend to increase ADG in the 2-stage weaned calves overall. It is possible that the 2-stage weaned calves were not as stressed compared to the abruptly weaned calves. This suggests that there could possibly be an interaction between ITM and level of stress. There was also a weaning strategy by ITM interaction for d0 to d124 feed efficiency where 2SAL steers' efficiency was increased and a

tendency for the same interaction from d42 to d124. These findings do not support our hypothesis that abruptly weaned calves receiving Multimin would experience the greatest positive response.

As mentioned previously, there were no weaning strategy by ITM interactions on cortisol levels found at either sample time during this study. Lefcourt et al. (1993) stated that diurnal variations in peripheral cortisol in cattle is 1 to 17 ng/mL. All cortisol concentrations, except for 2-stage Saline steers on d-7, were within this range suggesting that cattle were within the normal range of daily variations in stress. Further research is necessary for conclusive evidence about how weaning strategies and ITM affect cortisol levels in weaning and receiving cattle.

There was a weaning strategy by ITM interaction for the percent of steers walking on d 2 where AbtMM steers walked the most. Although authors expected abruptly weaned steers to spend more time walking after separation due to greater stress, it is difficult to explain why AbtMM steers walked more than AbtSAL steers.

There was only one instance of illness for the duration of this study. A 2MM calf was treated on d 18 for respiratory illness and was back to normal temp the next day. This extremely low rate in morbidity may be due to several factors; sufficient vaccination program, herd health, mild weather at time of weaning, and a well-balanced ration. A higher rate of morbidity may have been expected if any of these mentioned factors was not in-place or if there was more comingling from an increased number of sources. Roberts et al. (2016) found no difference in morbidity between calves given ITM and calves receiving none when the incidence was low, similar to the results of the current study. They did, however, find that bovine viral diarrhea-specific antibody response to a respiratory vaccine was greater for ITM calves. This suggests that

given more instances of morbidity, there could possibly be a response in favor of calves supplemented with ITM.

WEANING STRATEGY

There were no effects of weaning strategy on BW at any time throughout the entire study. Authors speculate that if calves were in a larger feedlot setting, abruptly weaned calves would have paced more and not have been in such close proximity to the feed bunk. This in turn could cause an increase in energy expenditure, a decrease in feed intake, and a decrease in BW for abruptly weaned calves. In a study by Burke et al. (2009) they found no difference in BW between calves weaned in two stages using nose clip and calves weaned by abrupt separation. Lippolis et al. (2016) found that after a 21 d period where 2-stage calves had nose clips inserted, control calves tended to have a greater BW 14 d and 21 d after separation from the dam. The current study's results do not follow this trend, but this may be due to the fact that 2-stage weaned calves did not have nose clips inserted as long. Two-stage weaning decreased the amount of shrink calves experienced compared to abrupt weaning. There were no other studies found that compared shrink between two-stage weaned calves and abruptly weaned calves. It seems likely that 2-stage weaned calves in the current study had decreased shrink due to less physical fill at the time of transport due to a decrease in ADG from d -6 to d 0, along with decreased amounts of the behavioral stress of vocalizing and walking upon arrival.

When evaluating other growth indicators, Haley et al. (2005) found that calves weaned in two stages had decreased ADG in the days while nose clips were present compared to calves that had free access to the dam's milk at that time. Two stage weaned calves did have, however, an increase ADG in the first week following terminal separation from the dam. Lippolis et al. (2016) also found that two stage weaned calves had a decreased ADG compared to abruptly

weaned calves in a 21 d period while calves had nose clips inserted preventing nursing. These findings are consistent with the current study where it was observed that 2-stage weaned calves had decreased ADG in the 6 d prior to separation, but had increased ADG in the 14 d following separation compared to abruptly weaned calves. Likely, the inability to nurse prior to separation caused a decrease in 2-stage weaned calves' ADG. In addition, a decrease in behavioral actions of stress post-separation of 2-stage weaned calves and/or an increase in behavioral actions of stress of the abruptly weaned calves post-separation caused a difference in ADG in favor of 2-stage weaned calves in the 14 d following separation. Similar to Haley et al. (2005) the current study shows an increase in the number of 2-stage weaned calves eating in the days following separation compared to abruptly weaned calves, which also likely contributed to this increase in ADG. Abruptly weaned calves had a greater ADG compared to 2-stage weaned calves from d 14 to d 28. This coincides with a decrease in DMI for 2-stage weaned calves during the same time frame. From d -6 to d 42 abruptly weaned calves had a greater ADG, which is supported by the fact that abruptly weaned calves had a greater G:F at two different time periods during this stage and a tendency for greater DMI from d 0 to d 42. Similar to the study by Haley et al. (2005) there was not an overall difference in ADG between weaning strategy treatment groups. They hypothesized that this was due to the fact that abruptly weaned calves spent more days nursing than 2-stage weaned calves. They hypothesized that results may have been different if cattle were considered weaned based on when milk was terminated as 2-stage calves had a greater ADG after abruptly weaned calves were also prohibited from nursing. Although the current study did show an increase in ADG for 2-stage weaned calves in the 2 weeks following separation from the dam, 2-stage weaned calves had a decreased ADG in the next 2 weeks which does not follow the same trend that Haley et al. (2005) found.

There were no weaning strategy effects on cortisol levels found in this study. Although using a different biomarker for stress, Burke et al. (2009) found no differences in leukocyte glutathione peroxidase activity between calves that were abruptly weaned and calves that were weaned in two stages suggesting weaning strategy had little influence over biomarkers of oxidative stress. To see if there are any differences in cortisol levels after the termination of milk for 2-stage weaned calves, it may be suggested in the future to sample cortisol levels on d -6. This would also allow researchers to see if there are any differences between two-stage weaned calves and abruptly weaned calves 6 d after the termination of milk for abruptly weaned calves.

The current study showed that 2-stage weaned steers vocalize less frequently than abruptly weaned steers on both d 1 and d 2. There was a lower percentage of 2-stage weaned steers standing and walking on d 1 compared to abruptly weaned steers. Additionally, 2-stage weaned steers spent more time lying on d 1 and eating on both d 1 and d 2 compared to abruptly weaned steers. This is similar to previous observations (Haley et al., 2001; Haley et al., 2005) that on d 2 and d 3 after separation that calves weaned in two stages with the use of anti-suckling nose flaps walk less, spend more time eating, more time lying and less time standing than calves weaned by traditional abrupt separation. However, on d 2, there was actually a greater percentage of 2-stage weaned steers standing on d 2. The combination of a greater percentage of 2-stage weaned steers eating on d 2 and the fact that abruptly weaned steers were visually fatigued on d2 as evidence of a greater percentage of abruptly weaned calves lying at that point could help explain this. These mentioned findings clearly demonstrate that two-stage weaned calves display less behavioral signs of stress upon separation from the dam than calves weaned by the traditional abrupt separation method. However, calves were not observed for behavior and

vocalizations during the 6 d while nose flaps were inserted in 2-stage weaned calves so there is no data that compared the treatment groups prior to separation.

INJECTABLE TRACE MINERALS

There was no effect of ITM on BW at any point in the study. Genther and Hansen (2012) found that during stressful events steers that consume a diet sufficient in trace minerals lost significantly less weight than steers consuming a diet deficient in trace minerals, which suggests that trace minerals may have a protective effect during times of elevated stress. Contrary to the previous study, Arthington et al. (2014) and Genther-Schroeder and Hansen (2015) found that calves receiving ITM actually had reduced ADG for a 14 d period following shipping and receiving. Also, Genther-Schroeder and Hansen (2015) found that ITM had minimal effects on overall growth performance of calves prior to and after transit and receiving. Roberts et al. (2016) found no effect of ITM on the BW and ADG of newly received feedlot cattle. There was no effect of Multimin on shrink in the current study. Genther-Schroeder and Hansen (2015) found that calves that received Multimin did not differ in BW shrink following a 20 h transit period compared to calves that received saline.

Multimin increased DMI from d 28 to d 42 and tended to increase it during the receiving period from d 0 to d 42. In the study by Genther-Schroeder and Hansen (2015) they found no differences in DMI nor G:F between calves that were administered saline and calves that were administered Multimin. Similarly, Roberts et al. (2016) saw no difference in DMI between saline injected calves and ITM injected calves, but noted that ITM injected calves tended to have a decreased G:F from d 28 to d 42.

There were no ITM effects on cortisol levels found in this study. Genther-Schroeder and Hansen (2015) found no differences in plasma cortisol levels following a 20 h transit period

between calves that received Multimin and calves that received saline. It has been shown that cattle that experience handling repeatedly have a decreased plasma cortisol concentration compared to calves that have not been handled (Cooke et al., 2009). This could help explain why the current study found no cortisol differences following trucking as calves had been handled in a working facility multiple times prior to blood sampling for plasma cortisol concentrations.

There were no effects of Multimin on the percent of steers standing lying and eating on neither d 1 nor d 2 and no effect of the percent of steers walking on d 1. No studies were found that compared the effects of ITM and calf behavior at weaning and receiving.

SUMMARY

In summary, weaning strategy had no effect on calf BW at any time. Two-stage weaning did decrease the amount of shrink occurred at shipping. There were variations in ADG among weaning strategies throughout the study but overall ADG was not affected. Injectable trace minerals were found to have minimal effects on calf performance throughout the trial but did have a tendency to increase overall ADG. There was an overall weaning strategy by ITM interaction on DMI where 2MM had the greatest DMI. Although 2-stage calves tended to have an increased G:F during the receiving period, there was no overall effect of weaning strategy nor ITM on feed efficiency. However, there was an overall weaning strategy by ITM interaction where 2SAL calves had increased feed efficiency. The effects of 2-stage weaning on calf behavior were clear and consistent. Two-stage weaned calves were visibly under less stress than abruptly weaned calves for the 2 d they were observed after separation from dam. Injectable trace minerals had minimal effects on calf behavior. Cortisol levels were not affected by weaning strategy nor ITM. The results of this study show that although 2-stage weaning decreases signs of behavioral stress at weaning; however, there needs to be further research conducted in order to

obtain conclusive evidence of the effects that 2-stage weaning and ITM have in conjunction with each other on calf performance and health at weaning.

TABLES

Table 1. Nutrient composition (DM basis) of diet fed to calves during receiving¹ and growing² period

Item	Inclusion, % DM	
	Transition	Growing
Ingredient, %		
Corn silage	55.0	15.0
High moisture corn	-	55.0
Modified wet distillers grains	20.0	20.0
Hay	15.0	-
Supplement ³	10.0	10.0
Analyzed nutrient content, %		
CP	13.1	13.6
NDF	39.1	19.0
ADF	20.6	7.4
Crude fat	5.1	4.2

¹Transition diet was fed to both heifers and steers from d 7 to d 42

²Growing diet was fed exclusively to steers from d 54 to d 124

³Supplement contained 76.2% ground corn, 15.9% limestone, 6.0% urea, 0.91% trace mineral salt (trace mineral salt = 8.5% Ca as CaCO₃, 5% Mg as MgO and MgSO₄, 7.6% K as KCl₂, 6.7% Cl as KCl₂ 10% S as S₈ [prilled], 0.5% Cu as CuSO₄ and Availa-4 [Zinpro Performance Minerals; Zinpro Corp, Eden Prairie, MN], 2% Fe as FeSO₄, 3% Mn as MnSO₄ and Availa-4, 3% Zn as ZnSO₄ and Availa-4, 278 mg/kg Co as Availa-4, 250 mg/kg I as Ca(IO₃)₂, 150 Se mg/kg Na₂SeO₃, 2,205 KIU/kg vitamin A as retinyl acetate, 662.5 KIU/kg vitamin D as cholecalciferol, 22,047.5 IU/kg vitamin E as dl- α -tocopheryl acetate, and less than 1% CP, fat, crude fiber, and salt), 0.155% Rumensin 90 (198 g monensin/kg Rumensin 90; Elanco Animal Health, Greenfield, IN), 0.1% Tylosin 40 (88 g tylan/kg Tylosin 40; Elanco Animal Health), and 0.75% soybean oil

Table 2. Effect of weaning strategy and Multimin inclusion on calf performance

Item	Abrupt		2-Stage		SEM	<i>P</i> -value		
	Multimin ¹	Saline	Multimin	Saline		Wean ²	ITM ³	W*ITM ⁴
BW, kg								
d -6	198	197	199	199	3.0	0.61	0.92	0.78
Pre-truck	209	208	204	205	3.2	0.23	0.96	0.75
Arrival	197	196	193	195	2.9	0.36	0.93	0.74
d 14	221	221	221	222	3.3	0.88	0.96	0.91
d 28	239	238	236	235	3.6	0.46	0.77	0.87
d 42	258	258	257	255	3.8	0.63	0.81	0.75
d 124 ⁵	404	405	413	399	7.3	0.83	0.38	0.29
Shrink ⁶ , %	5.73	5.35	4.88	5.08	0.213	0.01	0.67	0.17
ADG, kg/d								
Pre-Weaning								
d -6 to d 0	1.82	1.77	0.79	1.01	0.100	<0.01	0.39	0.17
Receiving								
d 0 to d 14	1.74	1.76	1.99	1.89	0.059	0.001	0.49	0.32
d 14 to d 28	1.23	1.25	1.10	1.03	0.090	0.05	0.79	0.63
d 28 to d 42	1.36	1.42	1.46	1.42	0.084	0.51	0.93	0.57
d 0 to d 42	1.45	1.47	1.51	1.44	0.031	0.57	0.34	0.10
d -6 to d 42	1.22	1.25	1.19	1.16	0.026	0.02	0.85	0.20
Growing								
d 42 to d 124	1.75	1.75	1.84	1.73	0.040	0.38	0.18	0.13
Overall								
d 0 to d 124	1.67	1.67	1.75	1.63	0.034	0.66	0.06	0.09
d -6 to d 124	1.58	1.59	1.63	1.52	0.033	0.80	0.14	0.06

¹ Multimin = Multimin 90 (MULTIMIN USA, Fort Collins, CO)²Wean = Main effect of weaning strategy³ITM = Main effect of injectable trace mineral⁴W*ITM = Weaning strategy × injectable trace mineral interaction⁵Only steers had weights taken at d 124⁶Shrink, % = calculated using the pre-truck BW and the arrival BW

Table 3. Effects of weaning strategy and Multimin inclusion on calf DMI and feed efficiency

Item	Abrupt		2-stage		SEM	<i>P</i> -value		
	Multimin ¹	Saline	Multimin	Saline		Wean ²	ITM ³	W*ITM ⁴
DMI, kg/d								
Receiving								
d 0 to d 14	4.45	4.54	4.62	4.47	0.10	0.62	0.78	0.26
d 14 to d 28	5.31	5.30	5.13	4.78	0.13	0.01	0.17	0.20
d 28 to d 42	5.83	5.68	5.78	5.30	0.15	0.15	0.04	0.27
d 0 to d 42	5.20	5.17	5.18	4.85	0.10	0.09	0.09	0.14
Growing								
d 42 to d 124 ⁵	7.92 ^b	8.20 ^{ab}	8.69 ^a	7.56 ^b	0.26	0.81	0.12	0.01
Overall								
d 0 to d 124	7.06 ^{ab}	7.21 ^{ab}	7.56 ^a	6.61 ^b	0.19	0.78	0.05	< 0.01
G:F								
Receiving								
d 0 to d 14	0.438	0.429	0.393	0.386	0.017	0.01	0.64	0.92
d 14 to d 28	0.258	0.241	0.213	0.214	0.022	0.12	0.73	0.69
d 28 to d 42	0.216	0.245	0.254	0.267	0.014	0.04	0.15	0.59
d 0 to d 42	0.281	0.284	0.295	0.299	0.008	0.08	0.67	0.93
Growing								
d 42 to d 124	0.222	0.211	0.214	0.231	0.007	0.39	0.68	0.06
Overall								
d 0 to d 124	0.240 ^{ab}	0.230 ^b	0.232 ^{ab}	0.247 ^a	0.006	0.425	0.684	0.05

¹Multimin = Multimin 90 (MULTIMIN USA, Fort Collins, CO)²Wean = Main effect of weaning strategy³ITM = Main effect of injectable trace mineral⁴W*ITM = Weaning strategy × injectable trace mineral interaction⁵Only steers had weights taken at d 124

Table 4. Effects of weaning strategy and Multimin inclusion on steer plasma cortisol level¹

	Abrupt		2-stage		SEM	<i>P</i> - value		
	Multimin ²	Saline	Multimin	Saline		Wean ³	ITM ⁴	W*ITM ⁵
Cortisol, ng/mL								
d -6	16.9	16.2	15.7	18.4	1.4	0.72	0.48	0.23
d 0	14.7	15.7	12.1	15.4	2.3	0.53	0.36	0.62

¹Blood samples for plasma cortisol analysis were taken exclusively on steers.

²Multimin = Multimin 90 (MULTIMIN USA, Fort Collins, CO)

³Wean = Main effect of weaning strategy

⁴ITM = Main effect of injectable trace mineral

⁵W*ITM = Weaning strategy × injectable trace mineral interaction

Table 5. Effect of weaning type and Multimin inclusion on steer behavior

Item	Abrupt		2-stage		SEM	<i>P</i> - value		
	Multimin ¹	Saline	Multimin	Saline		Wean ²	ITM ³	W*ITM ⁴
d 1 Behavior ⁵								
Standing, %	68.6	70.0	45.9	46.3	2.28	<0.01	0.69	0.83
Lying, %	31.4	30.0	54.1	53.7	2.28	<0.01	0.69	0.83
Walking, %	19.8	17.5	1.8	2.0	1.91	<0.01	0.61	0.52
Eating, %	16.3	19.7	30.4	29.9	1.75	<0.01	0.39	0.28
d 1 Vocalizations, calls/steer·hr ⁻¹	69	79	4	4	5.1	<0.01	0.36	0.31
d 2 Behavior ⁵								
Standing, %	49.3	48.0	53.4	53.5	1.95	0.02	0.76	0.73
Lying, %	50.7	52.0	46.6	46.5	1.95	0.02	0.76	0.73
Walking, %	9.7 ^a	5.2 ^b	2.1 ^c	1.7 ^c	0.75	<0.01	<0.01	0.01
Eating, %	19.2	24.8	35.5	34.5	1.85	<0.01	0.22	0.09
d 2 Vocalizations, calls/steer·hr ⁻¹	21	17	3	5	2.4	<0.01	0.61	0.29

¹Multimin = Multimin 90 (MULTIMIN USA, Fort Collins, CO)²Wean = Main effect of weaning strategy³ITM = Main effect of injectable trace mineral⁴W*ITM = Weaning strategy × injectable trace mineral interaction⁵d 1 Behavior = % of steers in pen observed for each behavioral category from 0800 to 1600

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